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(54) **Novel bacillus thuringiensis isolate denoted b.t. ps81gg, active against lepidopteran pests, and a gene encoding a lepidopteran-active toxin.**

(57) A novel B.t. isolate with activity against lepidopteran insects is disclosed. This isolate is highly active against the beet armyworm. A gene from this isolate has been cloned. The DNA encoding the B.t. toxin can be used to transform various prokaryotic and eukaryotic microbes to express the B.t. toxin. These recombinant microbes can be used to control lepidopteran insects in various environments.

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NOVEL BACILLUS THURINGIENSIS ISOLATE DENOTED B.t. PS81GG, ACTIVE AGAINST LEPIDOPTERAN PESTS, AND A GENE ENCODING A LEPIDOPTERAN-ACTIVE TOXIN

Background of the Invention

The most widely used microbial pesticides are derived from the bacterium Bacillus thuringiensis. This bacterial agent is used to control a wide range of leaf-eating caterpillars, and mosquitos. Bacillus thuringiensis produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. For example, B. thuringiensis var. kurstaki HD-1 produces a crystal called a delta toxin which is toxic to the larvae of a number of lepidopteran insects. The cloning and expression of this B.t. crystal protein gene in Escherichia coli has been described in the published literature (Schnepf, H.E. and Whitely, H.R. [1981] Proc. Natl. Acad. Sci. USA 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of B.t. crystal protein in E. coli.

The beet armyworm (BAW) Spodoptera exigua is a widely distributed noctuid moth that attacks a broad range of field and vegetable crops. This economically important species originated in Asia, but is now found in many parts of the world including the United States.

The plants attacked by BAW include beets, peanuts, alfalfa, lettuce, asparagus, tomatoes, potatoes, corn, onions, peas, cotton, citrus, mallow, and even certain wild grasses. It is also a pest on ornamentals and floriculture crops, such as carnations and chrysanthemums. Larvae will feed on the leaves, stems, buds, and sometimes the roots of host plants. Heavy infestations can lead to complete defoliation of fields of a crop, such as table beets.

The female oviposits egg masses of about 80 eggs on the host plant foliage. These egg masses are covered with hairs and scales from the body of the female. An average of 500 to 600 eggs may be deposited over a 4 to 10 day period. Larvae hatch in 2 to 5 days and begin feeding on the foliage. Young larvae will feed in growing tips of the plant and developing buds, while older larvae are less discriminating, feeding on older foliage as well. The five larval instars take about 3 weeks to complete, at which time the mature larva drops to the ground and pupates in the soil. In the warmer parts of its range the BAW passes through four generations per year.

This species is generally considered to be difficult to control in various crop situations. Methomyl (Lannate) is commonly used to control this pest in lettuce and other field crops. However, resistance to methomyl has been reported in populations exposed to heavy use of this chemical (Yoshida and Parella [1987]). Consequently, there is a need to develop alternative control strategies for this important pest.

Another aspect of the use of broad spectrum materials like Lannate for BAW control is secondary pest outbreaks. This is the disruptive influence of a non-selective chemical on natural control agents of other pests in a given crop. In tomatoes, chrysanthemums, and other crops, where leaf miners can be a problem, the use of Lannate severely depresses populations of the natural enemies of the leafminers. With removal of leafminer parasites, the leafminers can build to very high population levels and cause severe damage.

The discovery and use of a novel Bacillus thuringiensis isolate with good activity against BAW is a distinct improvement in the control of this lepidopteran pest.

Brief Summary of the Invention

The subject invention concerns a novel Bacillus thuringiensis isolate designated B.t. PS81GG which has activity against lepidopteran pests. It is highly active against the beet armyworm (BAW).

The subject invention also includes mutants of B.t. PS81GG which are also active against lepidopteran pests.

Also disclosed and claimed is the novel toxin gene from the novel isolate. This toxin gene can be transferred to suitable hosts via a plasmid vector.

Specifically, the invention comprises a novel B.t. isolate denoted B.t. PS81GG, and mutants thereof, and a novel delta endotoxin gene which encodes a 133,156 dalton protein which is active against lepidopteran pests.

Detailed Disclosure of the Invention

The novel toxin gene of the subject invention was obtained from a novel lepidopteran-active B. thuringiensis (B.t.) isolate designated PS81GG.

Characteristics of *B.t.* PS81GG

Colony morphology--Large colony, dull surface, typical B.t.
Vegetative cell morphology--typical B.t.
Flagellar serotype-- 3a3b, kurstaki.
Intracellular inclusions--sporulating cells produce a bipyramidal crystal which partially encloses a smaller cuboidal crystal.
Plasmid preparations--agarose gel electrophoresis of plasmid preparations distinguishes B.t. PS81GG from B.t. HD-1 and other B.t. isolates.
Alkali-soluble proteins--B.t. PS81GG has a 130,000 dalton protein and a 60,000 dalton protein.
Unique toxin--the 130,000 dalton toxin is different from any previously identified.
Activity--B.t. PS81GG kills all Lepidoptera tested, and is twice as active against Beet Armyworm as B.t. HD-1.
Beet Armyworm assay results:
B.t. PS81GG LC50 = 4 ug/ml
B.t. HD-1 LC50 = 8 ug/ml
Spodoptera exigua Bioassay: Dilutions are prepared of a spore and crystal pellet, mixed with USDA Insect Diet (Technical Bulletin 1528, U.S. Department of Agriculture), and poured into small plastic trays. Neonate Spodoptera exigua larvae are placed on the diet mixture and held at 25° C. Mortality is recorded after six days.

B. thuringiensis PS81GG, NRRL B-18425, and mutants thereof, can be cultured using standard known media and fermentation techniques. Upon completion of the fermentation cycle, the bacteria can be harvested by first separating the B.t. spores and crystals from the fermentation broth by means well known in the art. The recovered B.t. spores and crystals can be formulated into a wettable powder, a liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers and other components to facilitate handling and application for particular target pests. The formulation and application procedures are all well known in the art and are used with commercial strains of B. thuringiensis (HD-1) active against Lepidoptera, e.g., caterpillars. B.t. PS81GG, and mutants thereof, can be used to control lepidopteran pests.

A subculture of B.t. PS81GG and the E. coli host harboring the toxin gene of the invention, E. coli NRRL B-18428 was deposited in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, USA on October 19, 1988. The accession numbers are as follows:
B.t. PS81GG - NRRL B-18425; deposited October 11, 1988.
E. coli (pMYC388) - NRRL B-18428; deposited October 19, 1988.

The toxin gene of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of lepidopteran insects where they will proliferate and be ingested by the insects. The result is a control of the unwanted insects. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the B.t. toxin.

Where the B.t. toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes; fungi, particularly yeast, e.g., genera Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula, and Aureobasidium. Of particular interest are such phytosphere bacterial species as Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacterium tumefaciens, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioti, Alcaligenes entrophus, and Azotobacter vinlandii; and phytosphere yeast species such as Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces roseus, S. odorus, Kluyveromyces veronae, and Aureobasidium pollulans. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing the B.t. gene expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the toxin gene, the toxin gene under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In some instances, it may be desirable to provide for regulative expression of the toxin, where expression of the toxin will only occur after release into the environment. This can be achieved with operators or a region binding to an activator or enhancers, which are capable of induction upon a change in the physical or chemical environment of the microorganisms. For example, a temperature sensitive regulatory region may be employed, where the organisms may be grown up in the laboratory without expression of a toxin, but upon release into the environment, expression would begin. Other techniques may employ a specific nutrient medium in the laboratory, which inhibits the expression of the toxin, where the nutrient medium in the environment would allow for expression of the toxin. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The initiation and translational termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal.

In the direction of transcription, namely in the 5′ to 3′ direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5′ or 3′ of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second DNA sequence may be joined to the toxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototropy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the constructs, as well as for modifying the host. The organisms may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the toxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the toxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, and usually not more than about 1000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the toxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a toxin gene is lost, the resulting organism will be likely to also lose the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the trp gene, lac gene, gal gene, the lambda left and right promoters, the Tac promoter, the naturally-occurring promoters associated with the toxin gene, where functional in the host. See for example, U.S. Patent Nos. 4,332,898, 4,342,832 and 4,356,270. The termination region may be the termination region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system which is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus which is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pRO1614, and the like. See for example, Olson et al., (1982) J. Bacteriol. 150:6069, and Bagdasarian et al., (1981) Gene 16:237, and U.S. Patent Nos. 4,356,270, 4,362,817, and 4,371,625.

The B.t. gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiceae, such as Rhizobium; Spirillaceae, such as photobacterium, Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the B.t. gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as Rhodotorula sp., Aureobasidium sp., Saccharomyces sp., and Sporobolomyces sp.; phylloplane organisms such as Pseudomonas sp., Erwinia sp. and Flavobacterium sp.; or such other organisms as Escherichia, Lactobacillus sp., Bacillus sp., and the like. Specific organisms include Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis, Escherichia coli, Bacillus subtilis, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the B.t. toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results.

Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bio-availability or bioactivity of the toxin.

The cellular host containing the B.t. insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the B.t. gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The B.t. cells may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

The pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the lepidopteran pest(s), e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like.

Mutants of PS81GG can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of PS81GG. Other mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 Culturing *B.t.* PS81GG, NRRL B-18425

A subculture of B.t. PS81GG, NRRL B-18425, or mutants thereof, can be used to inoculate the following medium, a peptone, glucose, salts medium.

Bacto Peptone	7.5 g/l
Glucose	1.0 g/l
KH_2PO_4	3.4 g/l
K_2HPO_4	4.35 g/l
Salt Solution	5.0 ml/l
$CaCl_2$ Solution	5.0 ml/l
Salts Solution (100 ml)	
$MgSO_4.7H_2O$	2.46 g
$MnSO_4.H_2O$	0.04 g
$ZnSO_4.7H_2O$	0.28 g
$FeSO_4.7H_2O$	0.40 g
$CaCl_2$ Solution (100 ml)	
$CaCl_2.2H_2O$	3.66 g
pH 7.2	

The salts solution and $CaCl_2$ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30° C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The B.t. spores and/or crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

Example 2 - Cloning of Novel Toxin Gene and Transformation into *Escherichia coli*

Total cellular DNA was prepared by growing the cells of B. thuringiensis HD-1 and the novel B.t. PS81GG to a low optical density (OD_{600} = 1.0) and recovering the cells by centrifugation. The cells were protoplasted in TES buffer (30 mM Tris-Cl, 10 mM EDTA, 50 mM NaCl, pH = 8.0) containing 20 % sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4° C in 100 mM final concentration neutral potassium chloride. The supernate was phenol/chloroform (1:1) extracted twice and the DNA precipitated in ethanol. The DNA was purified by isopycnic banding on a cesium chloride gradient.

Total cellular DNA from PS81GG and HD-1 was digested with EcoRI and separated by electrophoresis on a 0.8% Agarose-TAE-buffered gel. A Southern blot of the gel was probed with the NsiI to NsiI fragment of toxin gene contained in the plasmid pM1,130-7 of NRRL B-18332 and the NsiI to KpnI fragment of the "4.5 Kb class" toxin gene (Kronstad and Whitely, [1986] Gene USA 43:29-40). These two fragments were combined and used as the probe. Results show that hybridizing fragments of PS81GG are distinct from those of HD-1. Specifically, a 3.0 Kb hybridizing band in PS81GG was detected instead of the 800 bp larger 3.8 Kb hybridizing band seen in HD-1.

Two hundred micrograms of PS81GG total cellular DNA was digested with EcoRI and separated by electrophoresis on a preparative 0.8% Agarose-TAE gel. The 2.5 to 3.5 Kb region of the gel was cut out and the DNA from it was electroeluted and concentrated using an ELUTIP™-d (Schleicher and Schuell, Keene, NH) ion exchange column. The isolated EcoRI fragments were ligated to LAMBDA ZAP™ EcoRI arms (Stratagene Cloning Systems, La Jolla, CA) and packaged using GIGAPACK GOLD™ extracts. The packaged recombinant phage were plated out with E. coli strain BB4 (Stratagene) to give high plaque density. The plaques were screened by standard nucleic acid hybridization procedure with radiolabeled probe. The plaques that hybridized were purified and re-screened at a lower plaque density. The resulting purified phage were grown with R408 M13 helper phage (Stratagene) and the recombinant BLUESCRIPT™ (Stratagene) plasmid was automatically excised and packaged. The "phagemid" was re-infected in XL1-Blue E. coli cells (Stratagene) as part of the automatic excision process. The infected XL1-Blue cells were screened for ampicillin resistance and the resulting colonies were analyzed by standard miniprep procedure to find the desired plasmid. The plasmid, pM4,31-1, contained an approximate 3.0 Kb EcoRI insert which contained an internal EcoRI site. The cloned fragment was sequenced using Stratagene's T7 and T3 primers plus a set of existing B.t. endotoxin oligonucleotide primers.

Total cellular PS81GG DNA was partially digested with AluI or RsaI and digests were mixed. DNA was modified with EcoRI methylase, EcoRI linkers were ligated onto ends, and excess linkers were removed by EcoRI digestion. DNA was size-fractionated on 0.8% Agarose-TAE gels and the approximately 4 to 8 Kb fragments were recovered by electroelution and NACS 52 column chromatography (BRL). Following insert ligation into LAMBDA ZAP™ (Stratagene) which was cut with EcoRI, DNA was packaged into phage heads. Libraries were screened by nucleic acid filter hybridization using a radiolabeled synthetic oligonucleotide probe (CCTGTCGGTTTTTCGGGGCC).

Hybridizing positives were plaque-purified and insert DNA was excised from phage DNA onto PBLUESCRIPT™ plasmid (Stratagene) with helper phage, according to manufacturers directions (Stratagene). The desired plasmid, pMYC388, was restriction mapped and the B.t. toxin coding sequence fully characterized by DNA sequencing.

Data from standard insect tests show that the novel B.t. PS81GG is active against all Lepidoptera tested.

The above cloning procedures were conducted using standard procedures unless otherwise noted.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. Also, methods for the use of lambda bacteriophage as a cloning vehicle, i.e., the preparation of lambda DNA, in vitro packaging, and transfection of recombinant DNA, are well known in the art. These procedures are all described in Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

The restriction enzymes disclosed herein can be purchased from Bethesda Research Laboratories, Gaithersburg, MD, or New England Biolabs, Beverly, MA. The enzymes are used according to the instructions provided by the supplier.

Plasmid pMYC388 containing the B.t. toxin gene, can be removed from the transformed host microbe by use of standard well-known procedures. For example, E. coli (pMYC388) NRRL B-18428 can be subjected to cleared lysate isopycnic density gradient procedures, and the like, to recover pMYC388.

Example 3 - Insertion of Toxin Gene Into Plants

The novel gene coding for the novel insecticidal toxin, as disclosed herein, can be inserted into plant cells using the Ti plasmid from Agrobacter tumefaciens. Plant cells can then be caused to regenerate into plants (Zambryski, P., Joos, H., Gentello, C., Leemans, J., Van Montague, M. and Schell, J [1983] Cell 32:1033-1043). A particularly useful vector in this regard is pEND4K (Klee, H.J., Yanofsky, M.F. and Nester, E.W. [1985] Bio/Technology 3:637-642). This plasmid can replicate both in plant cells and in bacteria and has multiple cloning sites for passenger genes. The toxin gene, for example, can be inserted into the BamHI site of pEND4K, propagated in E. coli, and transformed into appropriate plant cells.

Example 4 - Cloning of Novel *B. thuringiensis* Gene Into Baculoviruses

The novel gene of the invention can be cloned into baculoviruses such as Autographa californica nuclear polyhedrosis virus (AcNPV). Plasmids can be constructed that contain the AcNPV genome cloned into a commercial cloning vector such as pUC8. The AcNPV genome is modified so that the coding region of the polyhedrin gene is removed and a unique cloning site for a passenger gene is placed directly behind the polyhedrin promoter. Examples of such vectors are pGP-B6874, described by Pennock et al. (Pennock, G.D., Shoemaker, C. and Miller, L.K. [1984] Mol. Cell. Biol. 4:399-406), and pAC380, described by Smith et al. (Smith, G.E., Summers, M.D. and Fraser, M.J. [1983] Mol Cell. Biol. 3:2156-2165). The gene coding for the novel protein toxin of the invention can be modified with BamHI linkers at appropriate regions both upstream and downstream from the coding region and inserted into the passenger site of one of the AcNPV vectors.

The particular nucleotide sequence encoding the novel B.t. toxin gene, and the amino-acid sequence of the novel toxin are shown in claim 2.

It is well known in the art that the amino-acid sequence of a protein is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e. more than one coding nucleotide triplet (codon) can be used for most of the amino-acids used to make proteins, different nucleotide sequences can code for a particular amino-acid.

The novel B.t. toxin can be prepared via any nucleotide sequence (equivalent to that shown) encoding the same amino-acid sequence; the present invention includes such equivalent nucleotide sequences.

It has been shown that proteins of identified structure and function may be constructed by changing the amino-acid sequence, if such changes do not alter the protein secondary structure; see Kaiser, E.T. and Kezdy, F.J. (1984) Science 223:249-255. The present invention includes mutants of the amino-acid sequence depicted herein which have an unaltered protein secondary structure or, if the structure is altered, the mutant has the biological activity retained to some degree.

Claims

1. Bacillus thuringiensis PS81GG, having the identifying characteristics of NRRL N-18425, or a mutant thereof having activity against an insect pest of the order Lepidoptera.

2. DNA encoding a Bacillus thuringiensis toxin having the amino-acid sequence shown (in combination with a specific nucleotide sequence) below:

5 10 15 20
Met Asp Asn Asn Pro Asn Ile Asn Glu Cys Ile Pro Tyr Asn Cys Leu Ser Asn Pro Glu
ATG GAT AAC AAT CCG AAC ATC AAT GAA TGC ATT CCT TAT AAT TGT TTA AGT AAC CCT GAA

25 30 35 40
Val Glu Val Leu Gly Gly Glu Arg Ile Glu Thr Gly Tyr Thr Pro Ile Asp Ile Ser Leu
GTA GAA GTA TTA GGT GGA GAA AGA ATA GAA ACT GGT TAC ACC CCA ATC GAT ATT TCC TTG

45 50 55 60
Ser Leu Thr Gln Phe Leu Leu Ser Glu Phe Val Pro Gly Ala Gly Phe Val Leu Gly Leu
TCG CTA ACG CAA TTT CTT TTG AGT GAA TTT GTT CCC GGT GCT GGA TTT GTG TTA GGA CTA

65 70 75 80
Val Asp Ile Ile Trp Gly Ile Phe Gly Pro Ser Gln Trp Asp Ala Phe Leu Val Gln Ile
GTT GAT ATA ATA TGG GGA ATT TTT GGT CCC TCT CAA TGG GAC GCA TTT CTT GTA CAA ATT

85 90 95 100
Glu Gln Leu Ile Asn Gln Arg Ile Glu Glu Phe Ala Arg Asn Gln Ala Ile Ser Arg Leu
GAA CAG TTA ATT AAC CAA AGA ATA GAA GAA TTC GCT AGG AAC CAA GCC ATT TCT AGA TTA

105 110 115 120
Glu Gly Leu Ser Asn Leu Tyr Gln Ile Tyr Ala Glu Ser Phe Arg Glu Trp Glu Ala Asp
GAA GGA CTA AGC AAT CTT TAT CAA ATT TAC GCA GAA TCT TTT AGA GAG TGG GAA GCA GAT

125 130 135 140
Pro Thr Asn Pro Ala Leu Arg Glu Glu Met Arg Ile Gln Phe Asn Asp Met Asn Ser Ala
CCT ACT AAT CCA GCA TTA AGA GAA GAG ATG CGT ATT CAA TTC AAT GAC ATG AAC AGT GCC

145 150 155 160
Leu Thr Thr Ala Ile Pro Leu Leu Ala Val Gln Asn Tyr Gln Val Pro Leu Leu Ser Val
CTT ACA ACC GCT ATT CCT CTT TTG GCA GTT CAA AAT TAT CAA GTT CCT CTT TTA TCA GTA

165 170 175 180
Tyr Val Gln Ala Ala Asn Leu His Leu Ser Val Leu Arg Asp Val Ser Val Phe Gly Gln
TAT GTT CAA GCT GCA AAT TTA CAT TTA TCA GTT TTG AGA GAT GTT TCA GTG TTT GGA CAA

185 190 195 200
Arg Trp Gly Phe Asp Ala Ala Thr Ile Asn Ser Arg Tyr Asn Asp Leu Thr Arg Leu Ile
AGG TGG GGA TTT GAT GCC GCG ACT ATC AAT AGT CGT TAT AAT GAT TTA ACT AGG CTT ATT

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                        205                         210                         215                         220
Gly Asn Tyr Thr Asp Tyr Ala Val Arg Trp Tyr Asn Thr Gly Leu Glu Arg Val Trp Gly
GGC AAC TAT ACA GAT TAT GCT GTA CGC TGG TAC AAT ACG GGA TTA GAA CGT GTA TGG GGA

                        225                         230                         235                         240
Pro Asp Ser Arg Asp Trp Val Arg Tyr Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val
CCG GAT TCT AGA GAT TGG GTA AGG TAT AAT CAA TTT AGA AGA GAA TTA ACA CTA ACT GTA

                        245                         250                         255                         260
Leu Asp Ile Val Ala Leu Phe Pro Asn Tyr Asp Ser Arg Arg Tyr Pro Ile Arg Thr Val
TTA GAT ATC GTT GCT CTG TTC CCG AAT TAT GAT AGT AGA AGA TAT CCA ATT CGA ACA GTT

                        265                         270                         275                         280
Ser Gln Leu Thr Arg Glu Ile Tyr Thr Asn Pro Val Leu Glu Asn Phe Asp Gly Ser Phe
TCC CAA TTA ACA AGA GAA ATT TAT ACA AAC CCA GTA TTA GAA AAT TTT GAT GGT AGT TTT

                        285                         290                         295                         300
Arg Gly Ser Ala Gln Gly Ile Glu Arg Ser Ile Arg Ser Pro His Leu Met Asp Ile Leu
CGA GGC TCG GCT CAG GGC ATA GAA AGA AGT ATT AGG AGT CCA CAT TTG ATG GAT ATA CTT

                        305                         310                         315                         320
Asn Ser Ile Thr Ile Tyr Thr Asp Ala His Arg Gly Tyr Tyr Tyr Trp Ser Gly His Gln
AAC AGT ATA ACC ATC TAT ACG GAT GCT CAT AGG GGT TAT TAT TAT TGG TCA GGG CAT CAA

                        325                         330                         335                         340
Ile Met Ala Ser Pro Val Gly Phe Ser Gly Pro Glu Phe Thr Phe Pro Leu Tyr Gly Thr
ATA ATG GCT TCT CCT GTC GGT TTT TCG GGG CCA GAA TTC ACG TTT CCG CTA TAT GGA ACC

                        345                         350                         355                         360
Met Gly Asn Ala Ala Pro Gln Gln Arg Ile Val Ala Gln Leu Gly Gln Gly Val Tyr Arg
ATG GGA AAT GCA GCT CCA CAA CAA CGT ATT GTT GCT CAA CTA GGT CAG GGC GTG TAT AGA

                        365                         370                         375                         380
Thr Leu Ser Ser Thr Phe Tyr Arg Arg Pro Phe Asn Ile Gly Ile Asn Asn Gln Gln Leu
ACA TTA TCC TCT ACT TTT TAT AGA AGA CCT TTT AAT ATA GGG ATA AAT AAT CAA CAA CTA

                        385                         390                         395                         400
Ser Val Leu Asp Gly Thr Glu Phe Ala Tyr Gly Thr Ser Ser Asn Leu Pro Ser Ala Val
TCT GTT CTT GAC GGG ACA GAA TTT GCT TAT GGA ACC TCC TCA AAT TTG CCA TCC GCT GTA

                        405                         410                         415                         420
Tyr Arg Lys Ser Gly Thr Val Asp Ser Leu Asp Glu Ile Pro Pro Gln Asn Asn Asn Val
TAC AGA AAA AGC GGA ACG GTA GAT TCG CTG GAT GAA ATA CCA CCA CAG AAT AAC AAC GTG

                        425                         430                         435                         440
Pro Pro Arg Gln Gly Phe Ser His Arg Leu Ser His Val Ser Met Phe Arg Ser Gly Ser
CCA CCT AGG CAA GGA TTT AGT CAT CGA TTA AGC CAT GTT TCA ATG TTT CGT TCA GGC TCT

                        445                         450                         455                         460
Ser Ser Ser Val Ser Ile Ile Arg Ala Pro Met Phe Ser Trp Ile His Arg Ser Ala Glu
AGT AGT AGT GTA AGT ATA ATA AGA GCT CCT ATG TTC TCT TGG ATA CAT CGT AGT GCT GAA
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                    465                 470                 475                 480
Phe Asn Asn Ile Ile Ala Ser Asp Ser Ile Thr Gln Ile Pro Ala Val Lys Gly Asn Phe
TTT AAT AAT ATA ATT GCA TCG GAT AGT ATT ACT CAA ATC CCT GCA GTG AAG GGA AAC TTT

                    485                 490                 495                 500
Leu Phe Asn Gly Ser Val Ile Ser Gly Pro Gly Phe Thr Gly Gly Asp Leu Val Arg Leu
CTT TTT AAT GGT TCT GTA ATT TCA GGA CCA GGA TTT ACT GGT GGG GAC TTA GTT AGA TTA

                    505                 510                 515                 520
Asn Ser Ser Gly Asn Asn Ile Gln Asn Arg Gly Tyr Ile Glu Val Pro Ile His Phe Pro
AAT AGT AGT GGA AAT AAC ATT CAG AAT AGA GGG TAT ATT GAA GTT CCA ATT CAC TTC CCA

                    525                 530                 535                 540
Ser Thr Ser Thr Arg Tyr Arg Val Arg Val Arg Tyr Ala Ser Val Thr Pro Ile His Leu
TCG ACA TCT ACC AGA TAT CGA GTT CGT GTA CGG TAT GCT TCT GTA ACC CCG ATT CAC CTC

                    545                 550                 555                 560
Asn Val Asn Trp Gly Asn Ser Ser Ile Phe Ser Asn Thr Val Pro Ala Thr Ala Thr Ser
AAC GTT AAT TGG GGT AAT TCA TCC ATT TTT TCC AAT ACA GTA CCA GCT ACA GCT ACG TCA

                    565                 570                 575                 580
Leu Asp Asn Leu Gln Ser Ser Asp Phe Gly Tyr Phe Glu Ser Ala Asn Ala Phe Thr Ser
TTA GAT AAT CTA CAA TCA AGT GAT TTT GGT TAT TTT GAA AGT GCC AAT GCT TTT ACA TCT

                    585                 590                 595                 600
Ser Leu Gly Asn Ile Val Gly Val Arg Asn Phe Ser Gly Thr Ala Gly Val Ile Ile Asp
TCA TTA GGT AAT ATA GTA GGT GTT AGA AAT TTT AGT GGG ACT GCA GGA GTG ATA ATA GAC

                    605                 610                 615                 620
Arg Phe Glu Phe Ile Pro Val Thr Ala Thr Leu Glu Ala Glu Tyr Asn Leu Glu Arg Ala
AGA TTT GAA TTT ATT CCA GTT ACT GCA ACA CTC GAG GCT GAA TAT AAT CTG GAA AGA GCG

                    625                 630                 635                 640
Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Thr Asn Gln Leu Gly Leu Lys Thr Asn Val
CAG AAG GCG GTG AAT GCG CTG TTT ACG TCT ACA AAC CAA CTA GGG CTA AAA ACA AAT GTA

                    645                 650                 655                 660
Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Thr Tyr Leu Ser Asp Glu Phe Cys
ACG GAT TAT CAT ATT GAT CAA GTG TCC AAT TTA GTT ACG TAT TTA TCG GAT GAA TTT TGT

                    665                 670                 675                 680
Leu Asp Glu Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu
CTG GAT GAA AAG CGA GAA TTG TCC GAG AAA GTC AAA CAT GCG AAG CGA CTC AGT GAT GAA

                    685                 690                 695                 700
Arg Asn Leu Leu Gln Asp Ser Asn Phe Lys Asp Ile Asn Arg Gln Pro Glu Arg Gly Trp
CGC AAT TTA CTC CAA GAT TCA AAT TTC AAA GAC ATT AAT AGG CAA CCA GAA CGT GGG TGG

                    705                 710                 715                 720
Gly Gly Ser Thr Gly Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val
GGC GGA AGT ACA GGG ATT ACC ATC CAA GGA GGG GAT GAC GTA TTT AAA GAA AAT TAC GTC
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                    725                 730                 735                 740
Thr Leu Ser Gly Thr Phe Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu
ACA CTA TCA GGT ACC TTT GAT GAG TGC TAT CCA ACA TAT TTG TAT CAA AAA ATC GAT GAA

                    745                 750                 755                 760
Ser Lys Leu Lys Ala Phe Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
TCA AAA TTA AAA GCC TTT ACC CGT TAT CAA TTA AGA GGG TAT ATC GAA GAT AGT CAA GAC

                    765                 770                 775                 780
Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
TTA GAA ATC TAT TTA ATT CGC TAC AAT GCA AAA CAT GAA ACA GTA AAT GTG CCA GGT ACG

                    785                 790                 795                 800
Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly Glu Pro Asn Arg
GGT TCC TTA TGG CCG CTT TCA GCC CAA AGT CCA ATC GGA AAG TGT GGA GAG CCG AAT CGA

                    805                 810                 815                 820
Cys Ala Pro His Leu Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys
TGC GCG CCA CAC CTT GAA TGG AAT CCT GAC TTA GAT TGT TCG TGT AGG GAT GGA GAA AAG

                    825                 830                 835                 840
Cys Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn
TGT GCC CAT CAT TCG CAT CAT TTC TCC TTA GAC ATT GAT GTA GGA TGT ACA GAC TTA AAT

                    845                 850                 855                 860
Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu
GAG GAC CTA GGT GTA TGG GTG ATC TTT AAG ATT AAG ACG CAA GAT GGG CAC GCA AGA CTA

                    865                 870                 875                 880
Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu Ala Leu Ala Arg Val Lys
GGG AAT CTA GAG TTT CTC GAA GAG AAA CCA TTA GTA GGA GAA GCG CTA GCT CGT GTG AAA

                    885                 890                 895                 900
Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Glu Trp Glu Thr Asn Ile Val
AGA GCG GAG AAA AAA TGG AGA GAC AAA CGT GAA AAA TTG GAA TGG GAA ACA AAT ATC GTT

                    905                 910                 915                 920
Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Gln Leu
TAT AAA GAG GCA AAA GAA TCT GTA GAT GCT TTA TTT GTA AAC TCT CAA TAT GAT CAA TTA

                    925                 930                 935                 940
Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg
CAA GCG GAT ACG AAT ATT GCC ATG ATT CAT GCG GCA GAT AAA CGT GTT CAT AGC ATT CGA

                    945                 950                 955                 960
Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala Ile Phe Glu Glu
GAA GCT TAT CTG CCT GAG CTG TCT GTG ATT CCG GGT GTC AAT GCG GCT ATT TTT GAA GAA

                    965                 970                 975                 980
Leu Glu Gly Arg Ile Phe Thr Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn
TTA GAA GGG CGT ATT TTC ACT GCA TTC TCC CTA TAT GAT GCG AGA AAT GTC ATT AAA AAT
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                    985                 990                 995                1000
Gly Asp Phe Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu
GGT GAT TTT AAT AAT GGC TTA TCC TGC TGG AAC GTG AAA GGG CAT GTA GAT GTA GAA GAA

                   1005                1010                1015                1020
Gln Asn Asn Gln Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu
CAA AAC AAC CAA CGT TCG GTC CTT GTT GTT CCG GAA TGG GAA GCA GAA GTG TCA CAA GAA

                   1025                1030                1035                1040
Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr
GTT CGT GTC TGT CCG GGT CGT GGC TAT ATC CTT CGT GTC ACA GCG TAC AAG GAG GGA TAT

                   1045                1050                1055                1060
Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser
GGA GAA GGT TGC GTA ACC ATT CAT GAG ATC GAG AAC AAT ACA GAC GAA CTG AAG TTT AGC

                   1065                1070                1075                1080
Asn Cys Val Glu Glu Glu Ile Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Val
AAC TGC GTA GAA GAG GAA ATC TAT CCA AAT AAC ACG GTA ACG TGT AAT GAT TAT ACT GTA

                   1085                1090                1095                1100
Asn Gln Glu Glu Tyr Gly Gly Ala Tyr Thr Ser Arg Asn Arg Gly Tyr Asn Glu Ala Pro
AAT CAA GAA GAA TAC GGA GGT GCG TAC ACT TCT CGT AAT CGA GGA TAT AAC GAA GCT CCT

                   1105                1110                1115                1120
Ser Val Pro Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ser Tyr Thr Asp Gly Arg Arg
TCC GTA CCA GCT GAT TAT GCG TCA GTC TAT GAA GAA AAA TCG TAT ACA GAT GGA CGA AGA

                   1125                1130                1135                1140
Glu Asn Pro Cys Glu Phe Asn Arg Gly Tyr Arg Asp Tyr Thr Pro Leu Pro Val Gly Tyr
GAG AAT CCT TGT GAA TTT AAC AGA GGG TAT AGG GAT TAC ACG CCA CTA CCA GTT GGT TAT

                   1145                1150                1155                1160
Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu
GTG ACA AAA GAA TTA GAA TAC TTC CCA GAA ACC GAT AAG GTA TGG ATT GAG ATT GGA GAA

                   1165                1170                1175
Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
ACG GAA GGA ACA TTT ATC GTG GAC AGC GTG GAA TTA CTC CTT ATG GAG GAA
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3. DNA according to claim 2, having the said specific nucleotide sequence.

4. A toxin active against lepidopteran insects, having the amino-acid sequence shown in claim 2, or a mutant thereof which has an unaltered protein secondary structure and/or at least part of the biological activity.

5. A recombinant DNA transfer vector comprising DNA having all or part of the nucleotide sequence which codes for the amino-acid sequence shown in claim 2.

6. A DNA transfer vector according to claim 5, transferred to and replicated in a prokaryotic or eukaryotic host.

7. A microorganism capable of expressing a Bacillus thuringiensis toxin having the amino-acid sequence shown in claim 2.

8. A microorganism according to claim 7, which is a species of Pseudomonas, Azobacter, Erwinia, Serratia, Klebsiella, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter or Alcaligenes; a prokaryote selected from Enterobacteriaceae, Bacillaceae, Rhizobiaceae, Spirillaceae, Lactobacillaceae, Pseudomonadaceae, Azotobacteraceae and Nitrobacteraceae; or a lower eukaryote selected from Phycomycetes, Ascomycetes and Basidiomycetes.

9. A microorganism according to claim 7, which is Pseudomonas fluorescens or Escherichia coli.

10. A host according to claim 9, which is E. coli pMYC 388, having the identifying characteristic of

NRRL B-18428.

11. A microorganism according to claim 7, which is a pigmented bacterium, yeast or fungus.

12. A microorganism according to any of claims 7 to 11, which is pigmented and phylloplane-adherent.

13. Substantially intact cells of a unicellular microorganism according to any of claims 1 and 7 to 12, containing the toxin.

14. Cells according to claim 13, as obtained by treatment with iodine or other chemical or physical means to prolong the insecticidal activity in the environment.

15. A composition comprising a microorganism according to any of claims 1 and 7 to 14, e.g. as spores or crystals, in association with an insecticide carrier or with formulation ingredients to be applied as a seed coating.

16. A composition according to claim 15, wherein the carrier comprises beetle phagostimulants or attractants.

17. A method for controlling a lepidopteran insect pest, which comprises contacting the pest or its environment with a microorganism according to any of claims 1 and 7 to 14.

18. A method according to claim 17, wherein administration is to the rhizosphere, to the phylloplane, or to a body of water.

19. A method according to claim 17, which comprises placing a bait granule comprising the microorganism, e.g. as spores or crystals, on or in the soil when planting seed of a plant upon which the pest is known to feed.

20. A method according to claim 19, wherein the bait granule is placed at the same time as corn seed is planted in the soil.

21. A method according to claim 17, wherein the pest is present on stored products.

22. A method according to any of claims 16 to 21, wherein the pest is the beet armyworm.

23. Plasmid pMYC 388.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 31 0923

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 256 553 (SUMITOMO) ---		C 12 N 1/20 C 12 N 15/32 C 12 P 21/02 C 12 N 1/21 A 01 N 63/00
A	EP-A-0 186 379 (SUNTORY LTD) ---		
A	EP-A-0 200 708 (MONSANTO) ---		
A	EP-A-0 192 319 (MYCOGEN) ---		
A	EP-A-0 228 228 (MYCOGEN) -----		TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 N A 01 N C 12 P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23-11-1989	PULAZZINI A.F.R.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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